The paragraph in the specification beginning at line 24 of page 10 has been amended to recite that the exons are shown in blocks (*RBCS1B, RBCS2B,* and *RBCS3B*). Support for this amendment can be found, *e.g.,* in Figure 1. The legend to Figure 1 (page 10) explains that the noncoding genomic DNA of the *Arabidopsis thaliana RBSCB* locus is shown with the black line. The exons are therefore clearing indicated by the blocks.

Claim 1 has been amended to recite a heterologous nucleic acid molecule and identifying plant cells in which homologous recombination has occurred between the introduced heterolgous nucleic acid molecule and endogenous plant DNA. This amendment adds no new matter. Support for the amendment can be found, *e.g.,* in the application at page 11, lines 17-19.

For convenience, the rejections are addressed in the order presented in the Office Action mailed April 18, 2002.

Objection to the specification

The specification was objected to for referring to color figures on page 10, lines 24-32. The paragraph has been amended to delete references to colors, *e.g.,* green, orange, red, blue.

The objection further alleges that that the boundary of the luciferase gene is unclear in the figures. Applicants respectfully disagree. The legend to Figure 1B explains that in *ΔRBCS1B::LUC*, the *RBCS1B* sequences span from the PflMI site in *RBCS1B* exon 1 to the BsmI site in exon II. Accordingly, the luciferase-nos 3' terminator, which is cloned in frame to the truncated *RBCS1B* exon III, extends from the BsmI site, indicated by the "B", through the end of exon I of the *RBCS1B* region shown in Figure 1B. Thus, the boundary of the luciferase-nos-3' region is evident from the Figure and the description of the figure. Applicants therefore respectfully request withdrawal of the objection.

Rejection under 35 U.SC. § 112, second paragraph

Claims 1-12 were rejected as allegedly indefinite. The rejection alleges that claim 1 is indefinite in the recitation of a "polynucleotide sequence comprising a polypeptide sequence". Claim 1 has been amended to recite a heterologous nucleic acid molecule encoding a fusion protein. Applicants therefore request withdrawal of the rejection.

Rejection under 35 U.SC. § 112, first paragraph

Claims 4-6 and 8-12 were rejected as allegedly not enabled. The rejection alleges that the specification provides guidance only for a method of identifying homologous recombination using a selective reporter gene such as an NPTII gene, which confers kanamycin resistance. Further, the rejection alleges that no guidance is provided for obtaining the various types of homologous recombination events set forth in claims 8-10 and 12; or in inactivating an endogenous gene. Applicants respectfully traverse. The application provides a working example of a non-selective reporter gene and moreover, provides sufficient guidance for one of skill in the art to practice the claimed invention without undue experimentation.

The specification provides an example of a fusion protein comprising a non-selective reporter

The Examiner argues that the only working example provided for identifying homologous recombination is via the use of an NPTII gene. This argument appears to refer to claim 4. However, Applicants note that claim 4 is drawn to a method wherein the reporter sequence, which is a component of the fusion protein, is non-selective. The examples disclose a fusion protein comprising sequences encoded by *RBCSB1* fused to a luciferase reporter, which is, of course, non-selective. The NPTII gene that is included in the construct is not part of the fusion protein. Further, claim 4 does not preclude the inclusion of a selectable marker in the construct; it merely recites that the reporter sequence (in the fusion protein) is non-selective. Accordingly, the

Examiner's contention that only a selective reporter gene is disclosed in the examples is incorrect.

The specification provides guidance in identifying and screening plants in which a homologous recombination event occurs.

As the Examiner knows, the proper test of enablement is "whether one skilled in the art could make or use the claimed invention from the disclosure in the patent coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988); *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988); *see also* MPEP § 2164.01. According to Wands, the standard for undue experimentation is qualitative, not quantitative, in that "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should precede." *Wands*, 8 USPQ2d at 1404 (quoting *In re Jackson*, 217 USPQ 804 (Bd. Pat. App. & Int. 1982). Applicants assert that, in the present case, this requirement is easily met by the specification as filed. Given the disclosure in the specification along with the knowledge in the art of routine molecular biology, one of skill can practice the invention without undue experimentation.

Applicants have invented a method of identifying homologous recombination between an introduced nucleic acid and an endogenous gene in plants. The specification provides working examples and guidance to the practitioner in using routine molecular genetic techniques to perform this method. For example, the example section provides guidance for preparing internal recombinogenic substrate (IRS) constructs (page 16, line 16 through page 17, line 15); for transforming plants using the constructs (page 17, line 11-15); for identifying seedlings that are reporter (*e.g.*, LUC) positive (page 18, line 30 through page 19, line 2); and for analyzing genomic DNA from plants transformed with the IRS construct, *e.g.*, *ΔRBCS1B::LUC* fusion, to determine if a homologous recombination event occurred (page 20, line 3-13; page 20 line14 through page 21, line23). The techniques required to execute these methods are well known in

the art, *see, e.g.,* the specification at pages 13-15; and the references cited by the Examiner. In addition, these techniques can be used routinely to determine the "type" of homologous recombination. The Examiner provides no evidence or reasoning to suggest why a practitioner performing such routine techniques would not be expected to be able to identify the position and arrangement of the inserted sequence relative to the targeted gene, and thereby characterize the recombination event. Thus, a practitioner can readily use the methods of the invention and then, one by one, determine whether the recombination event in any particular plant is reciprocal or non reciprocal, and/or whether the homologous recombination leads to a nonequivalent cross-over event, inactivation of an endogenous gene, or whether it results in a recombinant allele. Therefore, in view of the guidance and examples provided in the specification, the claims are enabled. Applicants therefore respectfully request withdrawal of the rejection.

Rejection under 35 U.S.C. § 102(b)

Claims 1, 3, 4, and 8-12 were rejected as allegedly anticipated by Staub *et al.* The rejection alleges that Staub *et al.* teach a method for plant cell transformation using a construct comprising a promoterless fusion polynucleotide comprising a portion of the rbcL protein and the non-selectable beta-glucuronidase marker protein and further, that the transformed plants were screened for the production of the rbcL/GUS fusion protein produced by recombination. Applicants respectfully traverse. The reference does not anticipate the claimed invention as it does not disclose all of the elements of the claims.

Staub *et al.* describe a construct that has a promoterless chimeric *uidA* gene. However, the chimeric *uidA* gene is not a chimeric *rbcL uidA* gene, as suggested by the Examiner. The chimeric gene refers to the coding region of *uidA* fused to the 5' and 3' regulatory regions of the plastid gene (*see, e.g.,* page 847, column 2, lines 1-3 of the second paragraph under the section entitled "Experimental procedures".) Further, although the *rbcL* gene is included in the construct, the gene has it's own promoter (*see, e.g.,* lines 4-5 of the legend to Figure 1 on page 846.). Lastly, there are stop codons in all

three reading frames between the *rbcL* and *uidA* genes, and in addition, the *uidA* sequence includes a translation initiation site. (*see, e.g.,* page 847, column 2,first full paragraph, lines 7-11). Consequently, although a polycistronic mRNA may be produced, no fusion protein is produced. Thus, Staub *et al.* do not disclose each element of the claims. Accordingly, the reference is not anticipatory. Applicants therefore respectfully request withdrawal of the rejection.

Rejection under 35 U.S.C. § 102(e)

Claims 1-3, 7-10, and 12 were rejected as allegedly anticipated by Odell *et al.* (U.S. Patent 5,658,772). The rejection alleges that the patent teaches a gene encoding an NPT2 reporter protein that is fused with a portion of the LEU2 protein by virtue of the loxP site containing a portion of the *leu2* protein-encoding region. The rejection then asserts that the initial construct lacked sequences necessary for expression of the fusion polynucleotide by virtue of the presence of a polyadenylation site between the fusion polynucleotide transcription start site and the promoter. Applicants respectfully traverse. The reference does not disclose all of the elements of the claims and thus does not anticipate the claimed invention.

The Examiner argues that the lox P site contains a portion of the leu 2 protein-encoding region and would form a fusion protein with the NPT2 polypeptide sequence. However, there is no disclosure that the residual *leu* sequences in the construct described by the Examiner are capable of encoding a *leu* protein fragment (*see, e.g.,* column 21, lines 48-49, which discloses that part of the Leu2 gene was removed). Moreover, assuming *arguendo,* that there were such leu fragment-encoding sequences and that an RNA transcript comprising leu protein fragments was produced, there does not appear to be a translation initiation codon that would allow the fusion protein postulated by the Examiner to be produced (*see, e.g.,* column 21, lines 39-47). Thus, the patent does not disclose all of the elements of the claimed invention. Accordingly, it is not anticipatory. Applicants therefore respectfully request withdrawal of the rejection.

Rejections under 35 U.S.C. § 103

Claims 1-4, 7-10, and 12 were rejected as allegedly obvious over Swoboda *et al.* in view of Lyznik *et al.*. The rejection characterizes Swoboda *et al.* as teaching a method of identifying homologous recombination using a fusion protein comprising a portion of the GUS gene ligated to a portion of the CaMV open reading frame (ORF) V encoding the first 29 amino acids thereof. Lyznik is characterized as teaching a method for the identification of homologous recombination using a promoterless fusion polynucleotide construct comprising a GUS gene fused to an intron. The Examiner then alleges that it would have been obvious to use the fusion polynucleotide-mediated method of detecting homologous recombination in plant cells taught by Swoboda *et al.* and to modify that method by incorporating the promoterless construct taught by Lyznik *et al.* Applicants respectfully traverse.

In order to establish a *prima facie* case of obviousness, the rejection must demonstrate that: (1) there is some suggestion or motivation to modify the reference or combine the reference teachings; (2) there is a reasonable expectation of success: and (3) the prior art references suggest all the claim elements. *See, e.g.*, MPEP § 2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). Further, the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. (*see, In re Vaeck, supra*). These requirements are not satisfied by the rejection.

Swoboda et al. teach a *uidA* gene that is a fusion of the eukaryotic translation start site and the first 20 amino acis of the ORF V of CaMV with the bacterial *uidA* gene. However, the fusion protein sequences in the homologous recombination method of the invention comprises a polypeptide sequence of interest that targets an endogenous gene (*see, e.g.,* page 11, line 31 to page 12, line 5). Swoboda *et al.* does not teach such a sequence. The first 29 amino acids of the ORFv of CaMV does not target endogenous sequences in *Arabidopsis thalianaI.*

Furthermore, even if the cited references were to have taught all of the claim elements, the Examiner points to nothing in either reference that suggests

modification that would result in the claimed invention. Moreover, the rejection has failed to specifically identify the principles, known to one of ordinary skill in the art, that suggests the claimed invention (*In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998)). Lyznik *et al.* teach a co-transformation system using two plasmids which undergo homologous recombination. Why would one of skill be motivated to combine the plasmid system with Swoboda *et al.*? The rejection merely argues that one of skill would have been motivated to combine the cited art simply because individual elements in the claims are identified in each reference. This does not establish a proper motivation to combine references. Thus, in view of the deficiencies in the cited references and the failure to meet the requirements for establishing a case of obviousness, Applicants respectfully request withdrawal of the rejection.

Claims 1-10 and 12 were rejected as allegedly obvious over Swoboda *et al.* and Lyznik *et al.*, and further in view of Ow *et al.* Applicants respectfully traverse. Ow *et al.* disclose the use of a luciferase reporter in plants. The addition of the teachings in Ow *et al.* to the disclosures in Swoboda *et al.* and Lyznik *et al.* does not cure the defects in the combination of the primary references. Thus, a case of *prima facie* obviousness has not been established. Applicants therefore respectfully request withdrawal of the rejection.

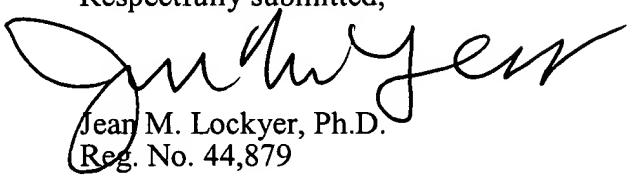
Claims 1-4 and 7-12 were rejected as allegedly obvious over Swoboda *et al.* and Lyznik *et al.*, and further in view of Miao *et al.* Applicants respectfully traverse. The primary references are defective for the reasons set forth above. Miao *et al.* do not cure the deficiencies. Further, as known in the art, the frequency of homologous recombination in plants is low (*see, e.g.,* Miao *et al.*, first paragraph of the introduction section). Even if the combination of references could be made, the rejection does not establish that one of skill could predict, with any reasonable expectation of success, that the method could be employed to identify homologous recombination between a promoterless fusion protein and an endogenous target. Thus, the disclosure in Miao *et al.* does not support a proper case of *prima facie* obviousness. Applicants therefore respectfully request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at .

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amendments to the specification:

Amendment to the paragraph starting on page 10, line 24 and continuing through page 11, line 4:

Figure 1A-1C show genetic constructs of the invention. Figure 1A shows the *Arabidopsis thaliana* RBCSB locus with black line indicating noncoding genomic DNA. Exons are shown in blocks [color: green, orange, and red] (*RBCS1B, RBCS2B,* [*and*] and *RBCS3B*, respectively). Exon size is not to scale but rather represents genic organization. Figure 1B shows an IRS construct used to make transgenic plants: NPTII gene shown in gray; *ΔRBCS1B::LUC* fusion consisted of *RBCS1B* sequences spanning from the PflMI site in *RBCS1B* exon I to the BsmI site in exon III; Firefly Luciferase nos 3' terminator [(blue)] is cloned in frame to the truncated *RBCS1B* exon III. *RBCS2B RBCS3B* sequences normally downstream of *RBCS1B* were placed 3' to the *ΔRBCS1B::LUC* fusion. Figure 1C shows a positive control construct (pJGJ204) consisting of a *RBCS1B* promoter *RBCS1B::LUC* gene fusion. Half arrows represent the approximate location and direction of oligonucleotide primers (o13 and o14) used in PCR reactions. Restriction enzymes: **P**, PflMI; **B**, BsmI; and **S**, SphI.

Claim amendments:

1. (amended) A method of identifying homologous recombination in plant cells, the method comprising:

contacting a plurality of plant cells with a heterologous nucleic acid molecule [comprising a fusion polynucleotide sequence comprising] encoding a fusion protein comprising a polypeptide sequence of interest linked to a reporter sequence, wherein the nucleic acid molecule lacks sequences necessary for expression of the fusion protein [polynucleotide sequence gene product] in a cell; and

detecting the presence of the [fusion polynucleotide sequence gene product] reporter activity in the plant cells, thereby identifying plant cells in which homologous recombination has occurred between the introduced heterologous nucleic acid molecule and endogenous plant DNA.

APPENDIX B

CLAIMS CURRENTLY UNDER EXAMINATION

1. (amended) A method of identifying homologous recombination in plant cells, the method comprising:

contacting a plurality of plant cells with a heterologous nucleic acid molecule encoding a fusion protein comprising a polypeptide sequence of interest linked to a reporter sequence, wherein the nucleic acid molecule lacks sequences necessary for expression of the fusion protein in a cell; and

detecting the presence of the reporter activity in the plant cells, thereby identifying plant cells in which homologous recombination has occurred between the introduced heterologous nucleic acid molecule and endogenous plant DNA.

2. The method of claim 1, wherein the step of contacting is carried out using a T-DNA vector.

3. The method of claim 1, further comprising the step of regenerating plants from the plant cells before the step of detecting the presence of the fusion sequence gene product.

4. The method of claim 1, wherein the reporter sequence is non-selective.

5. The method of claim 4, wherein the non-selective reporter sequence encodes luciferase.

6. The method of claim 5, wherein the step of detecting is carried out using video imaging equipment.

7. The method of claim 1, wherein the plant cell is Arabidopsis.

8. The method of claim 1, wherein the homologous recombination is reciprocal.

9. The method of claim 1, wherein the homologous recombination is non-reciprocal.

10. The method of claim 1, wherein the homologous recombination leads to a nonequivalent cross over event.

11. The method of claim 1, wherein the homologous recombination results in inactivation of an endogenous gene.

12. The method of claim 1, wherein the homologous recombination results in a recombinant allele.